

THE CLAIMS

What is claimed is:

1. A kit for performing specific binding assays with an optical unit including a light source positioned to direct light into an optical substrate and detection means oriented to detect light from a region proximal to the waveguide, which includes: a biosensor comprising:

a step gradient waveguide including a substrate formed of a first optical material of refractive index n_1 and having a surface disposed adjacent and in contact with a waveguide film formed of a second optical material having a refractive index n_2 which is greater than n_1 ; and at least one specific binding molecule immobilized to said waveguide film and constructed to bind with specificity an analyte.

2. The kit of Claim 1, wherein said second optical material is selected from the group consisting of: silicon oxynitride of refractive index n_2 , and deposited silicon dioxide; and said first optical material is selected from the group consisting of: deposited silicon dioxide, quartz or fused silica, silicon oxynitride of refractive index n_1 , and magnesium fluoride.

3. The kit of Claim 2, wherein said waveguide film is configured as a plurality of parallel longitudinal strips of said second optical material spaced from one another along said surface and constituting a plurality of waveguide channels, and further having a plurality of different species of said specific binding molecules immobilized to said waveguide film, with each of said waveguide channels having a different one of said species immobilized thereto.

4. The kit of Claim 2, wherein said substrate surface has a grating formed thereon, said grating being constructed to facilitate coupling of light from an incident excitation beam into said waveguide film.

5 5. The kit of Claim 1, wherein said waveguide film is coated with a coating which provides a level of nonspecific protein binding which is less than about 10% of the amount of specific binding of said analyte, said coating being selected from the group consisting of: polymethacryloyl polymer, polyethyleneglycol, and avidin; and wherein said capture molecules are immobilized to said waveguide film by binding to said coating.

10 6. The kit of Claim 1, wherein said waveguide is constructed by the steps of: providing a piece of the first optical material having a surface; vapor depositing the second optical material on said surface to a depth of between about 0.1 μm and about 10 μm to produce said waveguide film; coating at least one region of said waveguide layer with a resist compound which resists an etchant to produce a coated region and an uncoated region; and etching said waveguide layer with said etchant to remove said second optical material from said uncoated region.

15 7. The kit of Claim 6, wherein said second optical material is selected from the group consisting of: silicon oxynitride of refractive index n_2 , and silicon dioxide; and said first optical material is selected from the group consisting of: deposited silicon dioxide, quartz or fused silica, silicon oxynitride of refractive index n_1 , and magnesium fluoride.

20 8. The kit of Claim 6, in whose manufacture said step of coating at least one region with a resist comprises coating a plurality of parallel longitudinal strips along said surface with said resist thereby producing a plurality of waveguide channels, and wherein a plurality of different species of said specific binding molecules are immobilized to said waveguide film, with each of said waveguide channels having a different one of said species immobilized thereto.

9. The kit of Claim 8, whose construction further includes a step of etching a plurality of spaced grooves into said surface of said substrate prior to said step of depositing said waveguide layer, wherein said grooves are oriented substantially perpendicular to said waveguide channels, and spacing of said grooves is selected to serve as a grating facilitating efficient coupling of light from an incident excitation beam into said sensor strips.

10. The kit of Claim 1, wherein said second optical material is silicon oxynitride having an elemental ratio of approximately $\text{Si}_2\text{O}_3\text{N}$.

11. The kit of Claim 1, wherein said step gradient waveguide further includes a waveguide coupler contactingly disposed on said waveguide film, said waveguide coupler comprising:

an input waveguide formed of an optical material having a refractive index n_3 , and having a thickness which is between about 0.5 mm and about 5 mm; and a spacing layer formed of an optical material having a refractive index n_4 , wherein $n_4 < n_3$ and $n_4 < n_2$, and having a thickness selected to optimize the evanescent coupling of light from said input waveguide into said waveguide film.

12. Apparatus for performing evanescent immunofluorescence assays, including:

a composite waveguide comprising:

a substrate formed of a first optical material of refractive index n_1 and having two parallel planar surfaces separated by a thickness and a surrounding edge, said thickness being between about 1 μm and about 10 mm, and a waveguide film formed of a second optical material having a refractive index n_2 which is greater than n_1 and a thickness which is between about 0.1 μm and about 10 μm ;

a light source operably disposed to aim a beam into said composite waveguide for propagation by total internal reflection therein;

light coupling means operably associated with said composite waveguide for facilitating coupling of said light beam thereinto;

5 light collection means positioned to collect light that impinges on a plane spaced from said planar surfaces; and

detection means operably disposed with respect to said light collection means for detecting said collected light.

10 13. The apparatus of Claim 12, wherein said first and second optical materials are mutually selected from the group of pairwise combinations consisting of: deposited silicon dioxide/silicon oxynitride; quartz or fused silica/silicon oxynitride; silicon oxynitride of refractive index n_1 /silicon oxynitride of refractive index n_2 ; and magnesium fluoride/deposited silicon dioxide.

15 14. The apparatus of Claim 12, wherein said light coupling means is a waveguide coupler contactingly disposed on said waveguide film, and comprising: an input waveguide formed of an optical material having a refractive index n_3 , and having a thickness which is between about 0.5 mm and about 5 mm; and
20 a spacing layer formed of an optical material having a refractive index n_4 , wherein $n_4 < n_2$ and $n_4 < n_3$, and having a thickness selected to optimize the evanescent coupling of light from said input waveguide into said waveguide film.

25 15. The apparatus of Claim 13, wherein said waveguide film is constructed to have propagation losses in air of less than about 1 dB/cm.

16. An optical structure for performing specific binding assays with an optical unit comprising a light source positioned to direct light into the waveguide for

propagation by total internal reflectance therein and detection means oriented to detect light from a region proximal to the optical structure, said optical structure comprising: a substrate comprising a first optical material of refractive index n_1 and having two parallel planar surfaces separated by a thickness and a surrounding edge; said thickness being between about $0.3\ \mu\text{m}$ and about $10\ \text{mm}$; a waveguide film of a second optical material of refractive index n_2 in operable contact with at least a portion of one of said surfaces, said waveguide film having a thickness which is between about $0.3\ \mu\text{m}$ and about $5\ \mu\text{m}$; and coupling means integrally contacting said waveguide film for coupling light thereinto.

17. The optical structure of Claim 16, wherein said second optical material is selected from the group consisting of: silicon oxynitride of refractive index n_2 and silicon dioxide; and said first optical material is selected from the group consisting of: pure silicon dioxide, quartz or fused silica, silicon oxynitride of refractive index n_1 , and magnesium fluoride.

18. The optical structure of Claim 17, wherein said coupling means is selected from the group consisting of: an optical grating formed of said first material; an optical grating formed of said second material; and a waveguide coupler integrally mounted on said waveguide film, said waveguide coupler comprising an input waveguide constructed to receive light through an edge and to propagate the received light by total internal reflection, and a spacing layer interposed between said input waveguide and said waveguide film and having a thickness selected to optimize the evanescent coupling of light from said input waveguide into said waveguide film.

19. The optical structure of Claim 16, wherein said first optical material is quartz, fused silica or deposited SiO_2 , said coupling means is said waveguide coupler, and wherein said input waveguide is formed of an optical material selected from the group consisting of: rutile, zirconia, and high-index glass; and wherein said spacing

layer is formed of silicon dioxide and has a thickness of between about $0.1\ \mu\text{m}$ and about $5\ \mu\text{m}$.

20. The optical structure of Claim 16, wherein said waveguide film is configured as plurality of strips of said second optical material adhered to one of said surfaces in a spaced parallel array.

21. The optical structure of Claim 16, which further includes a plurality of sample wells formed thereon, said sample wells formed by the steps of:
epitaxially depositing an outer layer of refractive index n_1 upon said waveguide film;
applying a resist compound to a plurality of outlines which define said sample wells while leaving areas within and without the outlines uncoated; and
removing said outer layer by etching to expose said waveguide film in said uncoated areas.

22. A method of making a waveguide biosensor for performing specific binding assays with an optical unit including a light source positioned to direct light into the optical structure for propagation by total internal reflectance therein and detection means oriented to detect light from a region proximal to the optical structure, said optical structure constructed by the steps of:
providing a substrate comprising a first optical material of refractive index n_1 and having two parallel planar surfaces separated by a thickness and a surrounding edge;
depositing a waveguide layer of a second optical material having a refractive index n_2 on one of said surfaces, said waveguide layer having a thickness which is between about $0.1\ \mu\text{m}$ and about $10\ \mu\text{m}$ and n_2 being greater than n_1 ;
coating at least one region of said waveguide layer with a resist compound which resists an etchant to produce a coated region and an uncoated region;
etching said waveguide layer with said etchant to remove said second material from the uncoated region;

removing the resist compound from the etched waveguide to expose the coated region;
and
immobilizing a plurality of capture molecules to said exposed coated region, said
capture molecules being constructed to bind with specificity a selected analyte
molecule.

23. The method of Claim 22, further including a step of coating said exposed
coated region with a coating which provides a level of nonspecific protein binding
which is less than about 10% of the amount of specific binding of said analyte, said
coating being selected from the group consisting of: polymethacryloyl polymer,
polyethyleneglycol, and avidin; and wherein in said step of immobilizing capture
molecules, said capture molecules are bound to said coating.

24. The method of Claim 23, wherein said second optical material is selected
from the group consisting of: silicon oxynitride of refractive index n_2 , and deposited
silicon dioxide; and said first optical material is selected from the group consisting of:
deposited silicon dioxide, quartz or fused silica, silicon oxynitride of refractive index
 n_1 , and magnesium fluoride.

25. An immunofluorescence assay, comprising the steps of:
providing a waveguide which is optically conductive and which has at least one
surface having a plurality of capture molecules site-specifically immobilized
thereon, said capture molecules having a binding site which selectively binds a
selected analyte;
providing a light source operable to emit a light beam in a desired wavelength range
and positioned to send light into the waveguide;
providing detection means operably disposed for detecting fluorescence emitted from
the biosensor;
providing a sample comprising a buffer and a plurality of molecules of a selected
analyte;

providing a plurality of tracer molecules which are operable to emit fluorescence in response to stimulation by light from the light source;
combining the sample with the tracer molecules to produce a test solution;
placing the test solution in contact with the waveguide surface while operating said
5 light source to direct light into the waveguide; and
selectively detecting fluorescent light emitted from the tracer molecules.

26. The assay of Claim 25, wherein said step of providing a waveguide with site-specifically immobilized capture molecules includes the steps of:
10 coating the waveguide surface with a first coating to produce a coated surface;
providing a plurality of capture molecules;
modifying a single moiety which is the same on each capture molecule, to produce activated capture molecules having a modified moiety constructed to be coupled to the first coating; and
15 treating the coated surface with the activated capture molecules under conditions to cause the modified moiety to couple to the first coating and thereby immobilize the activated capture molecules to the waveguide surface.

27. The assay of Claim 25, wherein said first coating is selected from the group consisting of: avidin, a hydrogel formed of polymethacryloyl polymers, and a modified polyethylene glycol.

28. The immunofluorescence assay according to Claim 25, wherein an oligonucleotide primer, complementary to said analyte is immobilized to said
25 waveguide by amine-reactive, thiol-reactive, or (strep) avidin-biofin coupling chemistry.

29. The immunofluorescence assay according to Claim 25, wherein said tracer molecules are complementary to another sequence of said analyte.